IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Davis	
Application No.: 09/944,389	Group Art Unit: 1641
Filed: 9/4/2001	Examiner: Nguyen, Bao Thuy
Title: Analytical Test Device and Immuno Assays and Methods of Using Same	
Attorney Docket No.: IMIN.P-002-2	Confirmation No.: 9864

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER RULE 132

The undersigned, Balbir Raj, declares as follows:

1. I am an employee of Unipath Ltd., which along with Inverness Medical Switzerland GmBH, the assignee of the above-referenced application, is a subsidiary of Inverness Medical, Inc.

2. My Curriculum vitae is attached as Exhibit A. It shows my experience in the areas of immunochromatographic test strips and other analytical devices.

3. I am familiar with the above-captioned application, including the claims. The application relates to devices for assay of analytes such as human chorionic gonadotropin (hCG). The assays are lateral flow assays in which a labeled particulate reagent is carried through a porous carrier by application of a fluid sample to a macroporous body. If analyte of interest is present, the labeled reagent is retained at a test line. In the dry state prior to running the test, the labeled reagent is present within the macroporous body and is not present in the porous carrier.

Curriculum Vitae

Name: Mr Balbir Raj

Job Title: Technical Manager

Company: Unipath Ltd.

College Education

Higher National Certificate (HNC) 1984 - 1986
Paddington College
Edgeware
London

University Education

BSc (Hons) Upper Second Class, Applied Biology 1986 - 1990
University of Hertfordshire

Employment Details

During the 19 years of my employment at Unipath I have attained a thorough understanding of the scientific principles and techniques relating to immunoassay, in particular within the field of Lateral Flow Immunoassay.

I have experience in the design and construction of novel diagnostic products for consumer and professional use and have worked on the research and development of numerous products. These include tests in the area of pregnancy and fertility, (Clearblue Onestep, Clearplan Onestep, Clearblue One Minute, Persona) in addition to those for Infectious Diseases (Chlamydia and Infectious Mononucleosis) and assays for various haptens.

My role at Unipath demands the application of creativity and immunoassay expertise in the design, implementation and evaluation of novel product concepts, with the ability to transform these ideas into manufacturable products. In addition, I am engaged in identifying and implementing improvements to our existing technology, which will help maintain product performance that is superior to our competitors. My particular area of expertise lies in the research stage and entails the conception and bench testing of new ideas. This includes concepts that will provide the base platform that will ultimately supersede the Lateral Flow Technology.

I help support the Company's intellectual property by providing advice on technical issues relating to patent litigation. This involves discussions with lawyers and patent agents on the scientific and technical implications of our existing and future patent applications. I am involved in designing and performing experiments to provide evidence for the protection of the

Company's patents, as well as contributing constructive criticism on experiments performed by opposition parties in relation to our patents.

The knowledge gained from my work at Unipath has been focussed on sustaining strong intellectual property for the business. As such, publications of technical breakthroughs and novel concepts within the field I have worked in have been limited. I am not a member of any professional organisations.

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May 2004

UNIPATH

A comparison of lateral flow assay devices having the labelled capture reagent provided on a porous carrier with those devices having the labelled reagent provided on a separate macroporous carrier.

21st May 2004

A comparison of lateral flow assay devices having the labelled capture reagent provided on a porous carrier with those devices having the labelled reagent provided on a separate macroporous carrier.

Summary

Lateral flow assay devices were constructed wherein the particulate labelled binding reagent was provided in a dry porous carrier (one-part construct) and compared to those devices which were constructed with the reagent provided in a separate macroporous carrier (two-part construct).

The assays were then subjected to testing in order to evaluate and make a comparison between the two assay constructs.

Experimental outline

Apart from the provision of the labelled reagent in either the porous or the macroporous carrier, all other experimental parameters were kept as similar as possible.

Blue latex particles coated in anti-α hCG (an antibody capture reagent specific for the capture of the pregnancy hormone human chorionic gonadotropin (hCG)) were chosen as the direct particulate label, nitrocellulose membrane as the porous carrier and sintered plastic as the macroporous carrier.

One-part construct lateral flow assay devices were prepared by deposition of the labelled reagent directly onto the nitrocellulose porous carrier. Two-part construct lateral flow assay devices were prepared by deposition of the labelled reagent into a separate sintered plastic macroporous carrier, which was subsequently attached to a nitrocellulose porous carrier.

In both cases a second antibody (anti-β hCG) was immobilised onto the nitrocellulose porous carrier at a position downstream from the labelled reagent, in order to form the test-line.

The devices were subsequently packaged into separate hollow casings and an absorbent wick attached for sample collection. An absorbent sink was also provided at the distal end of the nitrocellulose part of the test strip. An aperture in the hollow casing provided a viewing window through which to observe the result at the test-line.

Devices prepared as above were tested by application of the analyte hCG prepared at various concentrations in aqueous buffer. The fluid sample was applied to the absorbent wick in order to generate a response from the assay device. Digital photographs of the test lines and of the release profile of the labelled reagent from the respective porous carrier and macroporous carrier were recorded and are shown in Figures 2 and 3.

Description of the figures

Figure 1 shows the construction of the one and two part construct lateral flow assay devices.

Figure 2 shows various scanned images of the nitrocellulose membranes following exposure to hCG buffer standards of different concentrations.

Figure 3 shows photographs taken at various time intervals of the viewing apertures of assay devices subjected to aqueous samples containing zero hCG analyte.

Figure 4 shows a graph of the test line intensity versus hCG concentration for the two assay formats.

Detailed description of the experiments

Control Measures

In order to ensure consistency between the one and two part constructs certain control features were put in place. These control measures ensured that parameters such as particle numbers, auxiliary components, (proteins etc), buffer, and fluid flow as well the offsets between the various zones were consistent. The measures included:

1. The two-part construct required the latex to be dried into a macroporous carrier, (sintered plastic). Drying into such a macroporous carrier requires additional reagents, (protein, sugar, detergent, EDTA and buffer) to be present during the drying process along side the latex. In order to ensure consistency, these buffers were also included in the latex that was deposited on the nitrocellulose membrane in the one part construct.

2. The void volume of the macroporous carrier within which the latex was dried as part of the two part construct was ~45µls per device, (the latex being present at 0.05% w/v solids). There was a need to concentrate the latex to higher % solids in order to deposit latex by airbrush onto the nitrocellulose membrane in the one part construct. The latex was hence concentrated by a factor of ~22.5 to give ~ 1.125% solids, of which ~2µls was applied to each test strip. In this manner the amount of latex applied to each construct was as similar as could be; also the latex was applied in the same buffer.

3. A macroporous carrier without latex was positioned upstream of the nitrocellulose membrane in the one part construct. With this in place, the dynamics of fluid flow would be consistent in the two constructs since the sample would encounter the same porous materials, (wick and macroporous carrier) on route to the test line present on the nitrocellulose membrane. The macroporous carrier without latex used in the one part construct contained the same protein, sugar, detergent, EDTA and buffer that were present in the macroporous carrier of the two-part construct. However, the concentration of these components present in the macroporous carrier of the one part system was reduced to account for the levels of reagents

that were present in the latex sprayed down on the nitrocellulose membrane. In this way, both constructs comprised equivalent quantities of reagents such as proteins, sugar etc.

4. The deposition of latex onto the nitrocellulose membrane produced a band that was ~9mm in width effectively making the label closer to the test line. To account for this, the test line position on nitrocellulose membrane used in the one piece construct was moved downstream to a position where it ensured that the offset between the latex and the test line was consistent between the two constructs, (this ensured distance x shown in figure 1 was consistent on both constructs).

Materials

- Blue latex particles 400nm in diameter, (2% solids w/v) prepared at Unipath
- Nitrocellulose membrane prepared with an immobilised zone of anti-β hCG. This membrane was blocked in polyvinyl alcohol, (PVA) after the immobilisation of the anti-β hCG reagent.
- Sintered plastic material 1.2mm thick treated in detergent in a buffer matrix.
- Latex drying buffer comprising 1% Bovine serum albumin, (BSA), 3% sucrose (w/v) plus 11.9mg/ml EDTA in 100mM Tris buffer pH 9.0
- Unipath wicks treated in Tris, Ultra wet & Tween 20.

A detailed list of materials is included in Appendix 1

M thodology

The methods in summary form are described below; however detailed descriptions of the methods and procedures are included in Appendix 2.

A) Airbrush deposition of latex onto nitrocellulose membrane, (one part construct).

The Unipath latex coated in anti-α hCG was placed into tubes and centrifuged to form a pellet. After removal and disposal of the supernatant, the pellet was resuspended into air drying buffer to give 1.125% solids (w/v).

Nitrocellulose membrane prepared as bands ~ 50mm wide by ~ 350mm in length were prepared with a zone of anti-β hCG and subsequently blocked in a mixture of PVA and sucrose. The Unipath latex above was deposited onto the membrane along its length at a deposition intensity of 0.29μls/mm using an airbrush. After deposition, the nitrocellulose was dried and stored desiccated ready for evaluation the following day. With 7mm wide test strips this equates to ~2μls of latex per test strip.

B) Infusion of latex into the macroporous carrier, (sintered plastic).

The stock of latex prepared at 1.125% solids (w/v) above was used; however this was diluted to 0.05% solids (w/v) in air-drying buffer. A sheet of sintered plastic, (treated in detergent and Tris buffer) was dipped into the diluted latex and allowed to soak in. After ~ 20 seconds, sheet was removed and dried in a heated tunnel with airflow. Once dry to touch, the infused sheet was sealed in a foil bag containing copious amounts of silica gel desiccant.

C) Infusion of drying buffer, (no latex) into the macroporous carrier, (sintered plastic) for use in the one part construct.

As explained in the section under Control Measures drying buffer was infused into sintered plastic material in the absence of latex particles. Here it was necessary to dilute the drying buffer to account for the amount of drying buffer that would already be present on the nitrocellulose component, (~2μls).
The treatment process was identical to that described above with the exception that latex particles were not included within the buffer.

Additional information with regard to absolute levels and dilutions of reagents are provided in Appendix 2.

D) Construction of test devices

One part construct.

The test strip construction is described in figure 1 section C. Here a section of macroporous carrier which did not contain latex particles, (7mm x 12mm) was overlapped onto a section of nitrocellulose, (7mm x 50mm). The nitrocellulose comprised an immobilised zone of anti-β hCG and had latex particles coated in anti-α hCG deposited onto it, upstream of the test line. Wick and paper sink materials were attached as shown and the construction was contained in a housing having a aperture for observation of the test line. The wick material protruded out of the casing to enable the application of the fluid sample.

Two part construct

The two-part construct was prepared as above except for the fact that the sintered plastic contained dried labelled reagent (figure 1 section D).

F) The evaluation of test devices in buffered hCG standards

The protruding section of the wicks on the test devices were held in buffered hCG standards for ~ 60 seconds prior to the device being laid flat on a bench and viewed for an additional 4 minutes. At this point each device was prised open and the nitrocellulose section of the test strip was removed and allowed to dry overnight at room temperature.

The following morning test line intensities were measured using an optical instrument allowing dose response profiles to be generated. The nitrocellulose membranes were mounted onto a card and scanned to produce a record of test line intensities.

Note: Buffer hCG standards tested: 0, 50, 100, 400mIU/ml

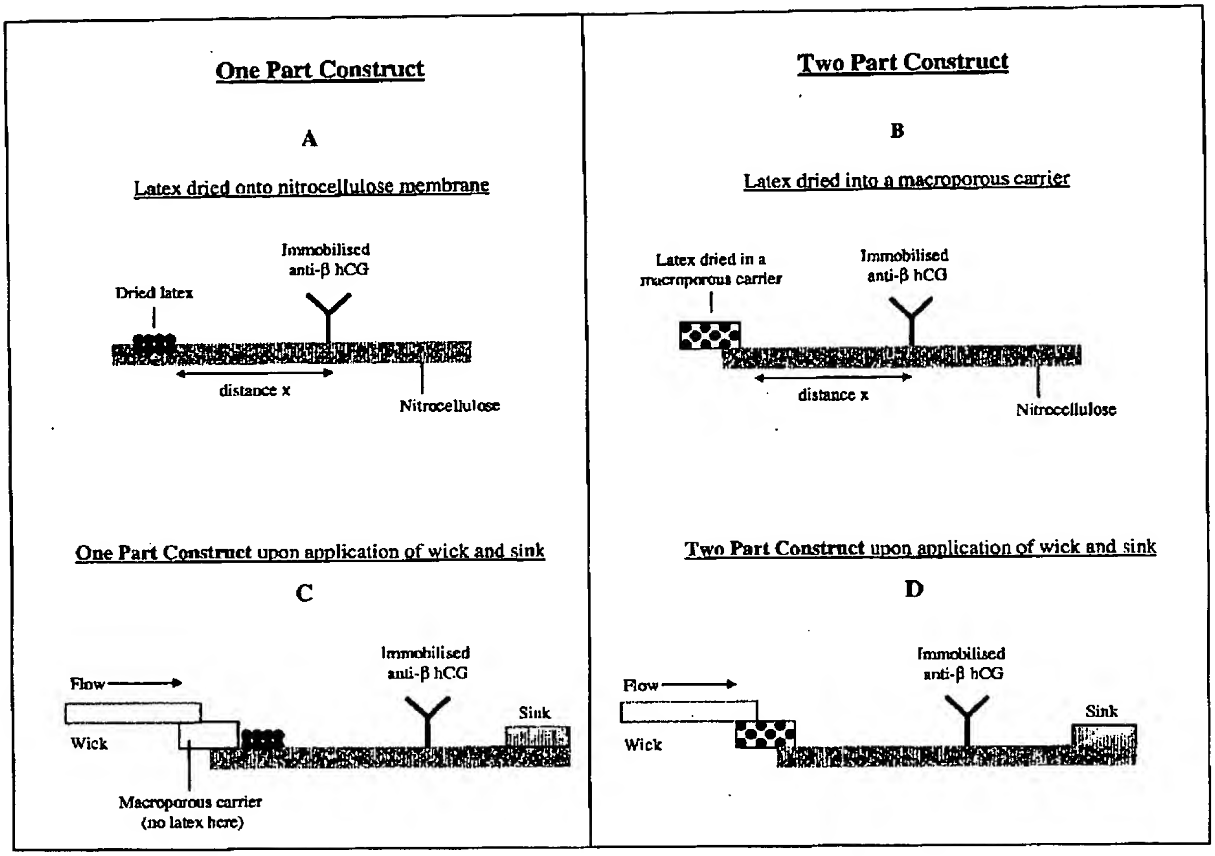
Additional devices were run with 0 hCG standard and photographs of the observation aperture were taken at specified times after wetting became apparent in the test window.

Results and Conclusions

Figure 3 shows that when the labelled reagent is provided in the macroporous carrier it releases upon wetting in a controlled, uniform manner. From the photographs taken at the observation aperture it may be seen that streaking of the labelled reagent does not occur. By contrast, when the labelled reagent is provided in the porous carrier an uncontrolled release of the labelled reagent occurs upon wetting with the fluid sample resulting in streaking of the reagent. This may lead to confusion for the user as well as result in the formation of incomplete or broken test lines.

From Figures 2 and 4 it may be seen that superior signal intensity is obtained at various analyte concentrations on using assay devices with the labelled reagent provided in the macroporous carrier. Provision of the labelled reagent in the macroporous carrier leads to improved reagent release which results in an increased binding efficiency between the labelled capture reagent and analyte. By comparison, the labelled reagent provided on the porous carrier tends to release reagent into the sample fluid in an uncontrolled fashion. This results in a less efficient mixing between analyte and labelled reagent resulting in reduced assay sensitivity.

Figure 1



One Part Construct
A
Latex dried onto nitrocellulose membrane
Immobilised anti-β hCG
Dried latex
distance x
Nitrocellulose
Two Part Construct
B
Latex dried into a macroporous carrier
Latex dried in a macroporous carrier
Immobilised anti-β hCG
distance x
Nitrocellulose
One Part Construct upon application of wick and sink
C
Immobilised anti-β hCG
Flow
Sink
Wick
Macroporous carrier (no latex here)
Two Part Construct upon application of wick and sink
D
Immobilised anti-β hCG
Flow
Sink
Wick

Figure 2

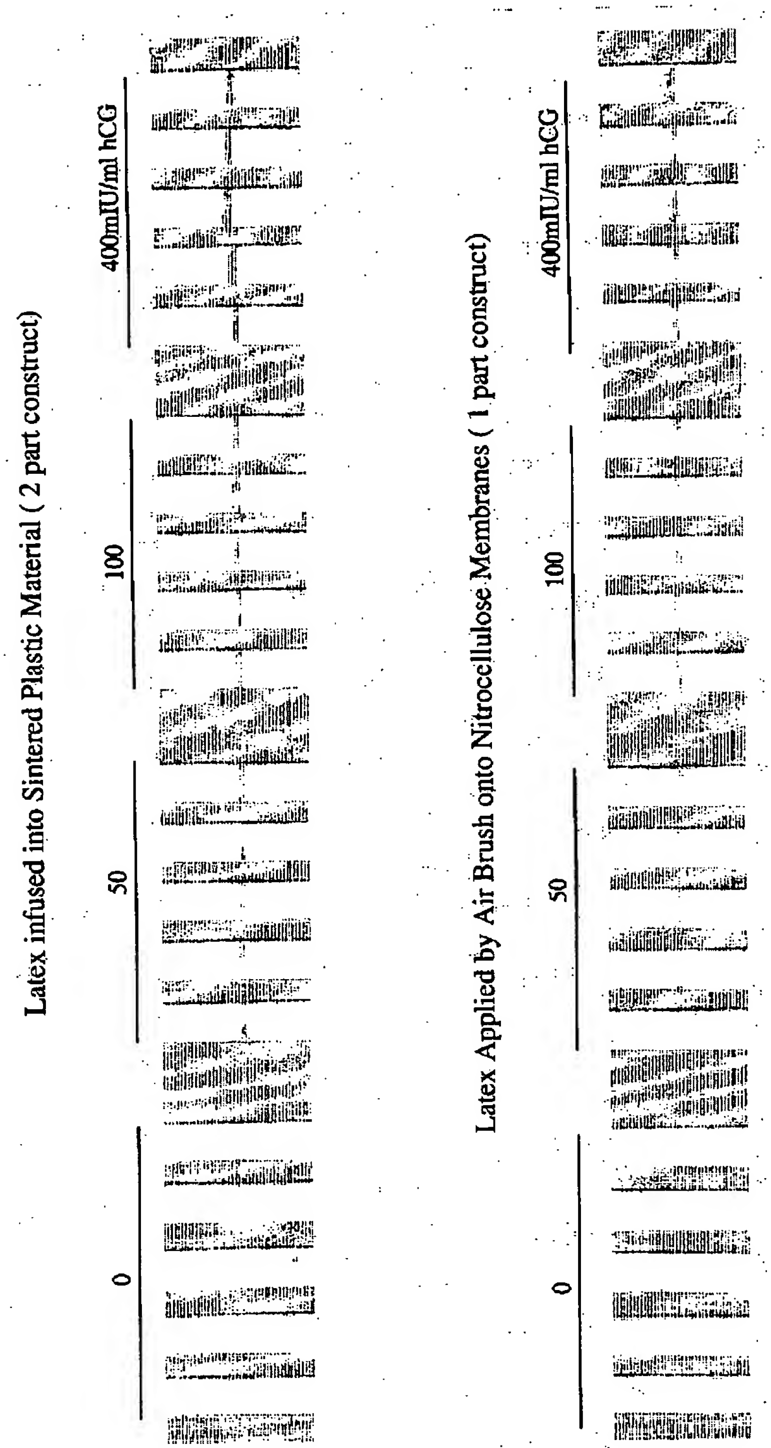
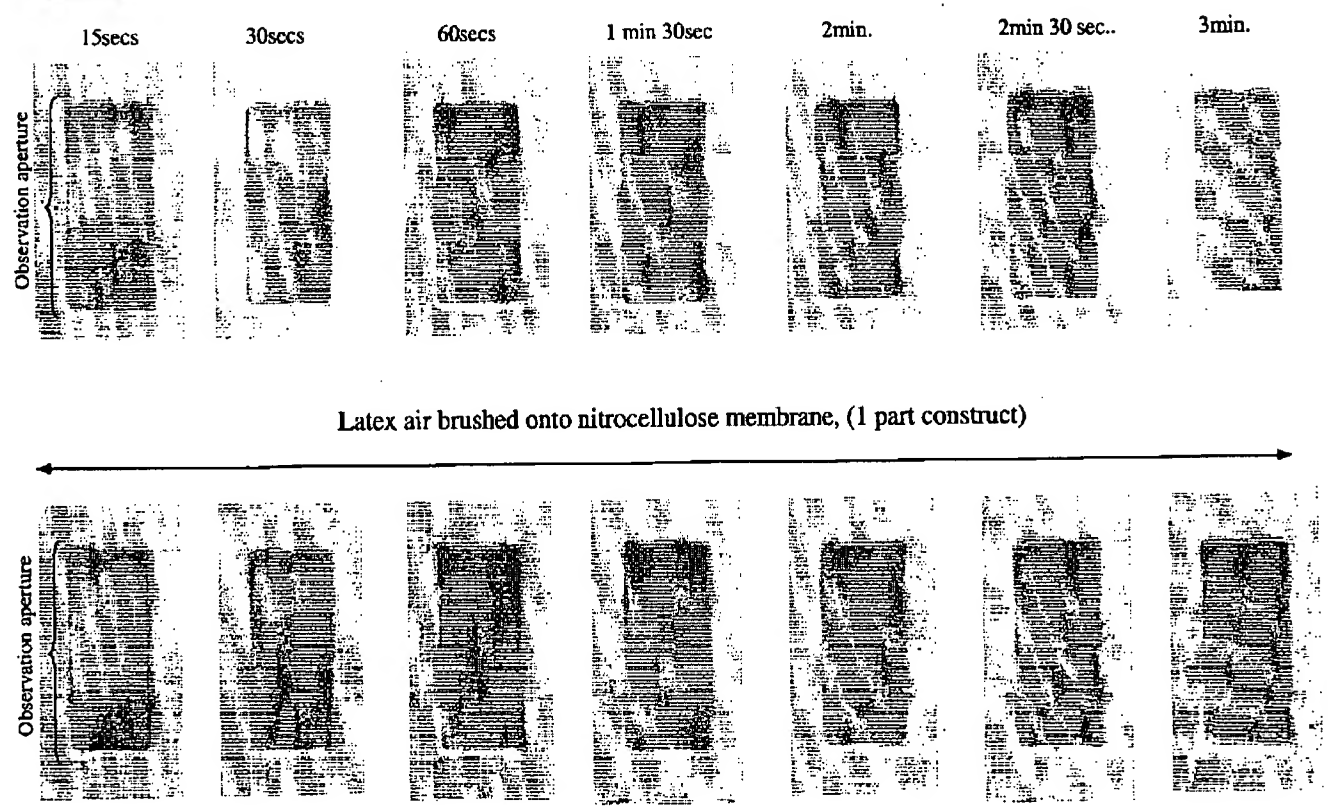

Latex infused into Sintered Plastic Material (2 part construct)
400mIU/ml hCG
100
50
0
Latex Applied by Air Brush onto Nitrocellulose Membranes (1 part construct)
400mIU/ml hCG
100
50
0

Figure 3

A comparison of latex sprayed onto nitrocellulose membrane with latex dried in a macroporous carrier

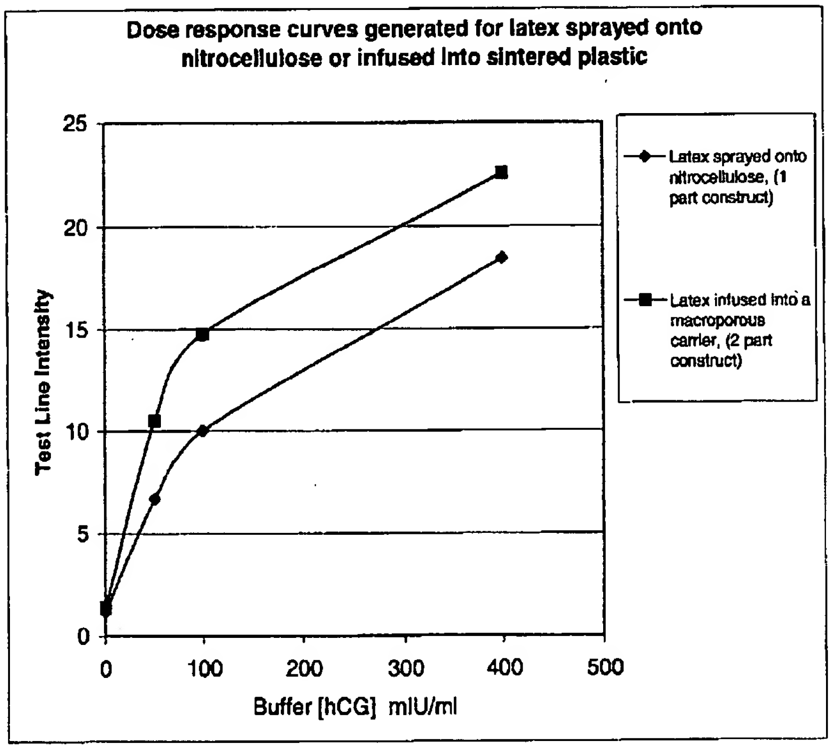
Latex infused into a macroporous carrier, (2 part construct)

Latex air brushed onto nitrocellulose membrane, (1 part construct)



Devices run in 0 hCG buffer standard and photographed at set times

Figure 4



Raw data - Optical readings of test line intensities using Unipath's camera system

<u>Latex sprayed onto nitrocellulose membrane</u>
<u>(One Part Construct)</u>

	0	50	100	400mIU/ml hCG
	1.35	6.39	9.26	18.54
	1.27	7.18	10.67	17.78
	1.16	5.73	10.99	19.02
	1.17	7.60	9.56	18.54
	1.19	6.39	9.53	18.46
mean	**1.23**	**6.66**	**10.00**	**18.47**

<u>Latex infused into sintered plastic</u>
<u>(Two Part Construct)</u>

	1.40	8.89	15.41	18.40
	1.26	11.45	15.33	24.25
	1.37	10.70	14.73	22.20
	1.35	10.19	15.03	24.09
	1.55	11.35	13.37	23.91
mean	**1.39**	**10.51**	**14.77**	**22.57**

Appendix 1

Materials

- Duke Scientific blue latex CB1587-1 coated in 3299 anti-α hCG @ 133μg/ml in 100mM borate buffer pH 8.5. Latex coated at 2% solids and supplied as 2% solids (w/v) in 100mM borate buffer pH 8.5 .Batch CV562 120504 Doc. No. CVII Latex/402.3

- Schleicher & Schuell nitrocellulose membrane batch CU1295-2

- 2.5mg/ml 3468 anti-β hCG in PBSA batch PL1197 (for deposition onto the nitrocellulose membrane.

- 1% Airvol 9K Polyvinyl alcohol (PVA) batch 18034004 plus 3% Sucrose (BDH Analar K30139086 236) both w/v prepared in deionised water.

- Sintered porous plastic 1.2mm thick from Sintair batch 11811, treated in 0.15M Tris pH 8.1 plus 8% (v/v) Decon 90. Batch CV562 Doc. No. Pad Wash/301.1

- Latex drying buffer 100mM Tris pH 8 plus 1% Decon 90 (v/v), 3% BSA (Intergen W22903), 1% sucrose, (both w/v) and 11.9mg/ml EDTA. Batch CV562 Doc. No. CVII Latex / 403.3

- Buffered hCG standards, Unipath 0, BNEG/328, 50mIU/ml B50/415, 100mIU/ml B100/412 and 400mIU/ml B400/412

- Unipath wick batch 9871 for use in CBIII

Appendix 2

Methods

A) Airbrush deposition of latex onto nitrocellulose membrane, (one part construct).

1. Centrifuge 7mls of latex @ 2% solids (w/v) coated in anti-α hCG in a Heraeus Biofuge 17RS centrifuge for 5 minutes at 17,000 rpm at 15°C.

2. Remove and discard the supernatant and resuspend the pellet into 12.44mls of latex drying buffer to give 1.125% (w/v) solids latex.

3. Deposit the latex onto nitrocellulose membrane prepared with a zone of anti-β hCG, (note the nitrocellulose membrane was blocked). Deposition intensity = 0.29μls/mm. The deposition was performed by air brush with an offset of ~ 3mm from the edge of the membrane, upstream of the test line.

4. Dry the membrane by in a tunnel drier set at 55°C belt speed 5.0 (Headinair). Store the membrane in foil bags with desiccant.

B) Infusion of latex into the macroporous carrier, (sintered plastic).

1. Dilute the latex from section A2 above to produce 0.05% solids latex (w/v) using latex drying buffer. Mix 4.4mls of latex @ 2% solids with 95.6mls of latex drying buffer.

2. Immerse sintered pad material into the above latex and let it soak in for ~ 20 seconds. Remove the sintered material and allow this to dry in a Headinair tunnel drier set at 55°C on speed 0. Pass the material trough the dryer twice. Store the material in a sealed foil bag with desiccant.

C) Infusion of latex-drying buffer, (no latex) into the macroporous carrier, (sintered plastic) for use in the one part format.

As protocol B above, however there was no latex in the drying buffer. On dilution of the air-drying buffer, the volume of latex used in B above was replaced with deionised water.

Balbir Raj

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